PATENT SPECIFICATION

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COMPLETE SPECIFICATION

Hemostatic preparations and process for the manufacture thereof

We, FARBWERKE HOECHST AKTIENGESELLSCHAFT VORMALS MEISTER LUCIUS & BRÜNING, a German Company, of Frankfurt(M)-Höchst, Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

At the present time highly active thrombin preparations are without doubt the best hemostatics for local application. They have become indispensable for numerous therapeutic purposes. Since they are prepared from the blood of man and animals and only moderate yields are obtained, these thrombin preparations are expensive products. Moreover, the action of thrombin is impeded in certain cases by antithrombins, either therapeutically administered (heparin or the like) or contained in the blood (for instance, icterus antithrombin).

The present invention is based on the observation that the action of thrombin preparations can be increased many times and at the same time their stability enhanced, by reacting the preparation in aqueous solution with an acridine compound containing a basic substituent in the 9-position, for example, 2-ethoxy-6:9-diamino-acridine lactate, 2-ethoxy-9-[para-(γ-diethylamino - β - hydroxypropylamino) - phenylamino] - acridine trihydrochloride, 3-nitro-9-(γ-diethylamino-β-hydroxypropylamino)-acridine dihydrochloride, 2-ethoxy-9-ethylamino-acridine hydrochloride, 2-ethoxy-9-aminoacridine methochloride, 2-ethoxy-9-(para-hydroxyphenyl-ethylamino)-acridine glycollate or 2-ethoxy-9-[1^1-phenyl - 2^1:3^1 - dimethyl-pyrazolonyl-(4^1)]-amino-acridine hydrochloride.

The basic acridine derivatives appear to react with the acid groups of thrombin with formation of complex compounds of increased activity and of substantially lower sensitivity to the aforesaid antithrombins.

It has also been found that the hemostatic activity of the thrombin preparations obtainable by the present process can be still further increased by the addition of substances known to accelerate the action of thrombin, such as protamines, gelatin, starch, thrombocyte extracts or polyvinyl pyrrolidone, and also pectin or pectin derivatives and alginates or their derivatives.

The following experiment exemplifies the increased activity resulting from the reaction of thrombin with the above mentioned acridine derivatives:

EXPERIMENT 1.

The periods required for the coagulation of plasma were determined with various quantities of a thrombin preparation, with and without previous reaction with 2-ethoxy-6:9-diamino-acridine lactate. 0.2 cc. portions of oxalated plasma from cattle, which had been freed from prothrombin by adsorption with barium sulphate, were mixed at 37° C. with a mixture of the thrombin preparation dissolved in 0.2 cc. of water, on the one hand, with 0.1 cc. of water, and, on the other, with 0.1 cc. of a solution of 2-ethoxy-6:9-diamino-acridine lactate of 0.1 per cent. strength, and the coagulation periods were determined with the stop watch. The results are set out in the following Table:—

[*Price* 2/8]

Quantity of the thrombin preparation in micrograms	Coagulation period in seconds	
	without the acridine compound	after reaction with the acridine compound
800	4	immediately
400	4	"
200	5.5	"
100	7.5	4.5
50	12	6
25	19.5	8.5
12.5	33	10

The above data show that by the use of 2-ethoxy-6:9-diamino-acridine lactate, applied within a very wide range of concentration, a considerably increased action of thrombin is produced. A similar shortening of the coagulation period is obtained by using, instead of 2-ethoxy-6:9-diamino-acridine lactate, for example, one of the following acridine derivatives: 3 - ethoxy - 9 - [para - (γ - diethylamino - β - hydroxypropyl - amino) - phenylamino] - acridine trihydrochloride, 3 - nitro - 9-(γ - diethylamino-β-hydroxypropylamino) - acridine dihydrochloride, 2 - ethoxy - 9 - ethylamino - acridine hydrochloride, 2-ethoxy-9-amino-acridine methochloride, 2-ethoxy - 9 - (parahydroxyphenyl - ethylamino) - acridine glycollate and 2-ethoxy-9 - [1^1 - phenyl - 2^1:3^1 - dimethyl-pyrazolonyl - (4^1)] - amino - acridine hydrochloride.

The quantity of the various acridine derivatives necessary to produce an optimum effect as regards increased action and stability depends for any given thrombin preparation on the process by which the latter was prepared and its degree of purity, and can be determined in any particular case by means of simple experiments. Obviously there is no need to use only the optimum proportion, since a useful effect can be obtained with a larger or smaller proportion. It will therefore be understood that the proportions mentioned in the Examples are merely illustrative and are not to be taken as limitative.

When natural blood is used similar results are obtained. It was found that the thrombin activity can be increased about 4 to 5 times.

The preparations obtained by the reaction of thrombin with acridine compounds are not only more potent but, as has already been stated, are substantially less sensitive to antithrombins.

The following experiment shows that the action of heparin is suppressed by reaction products of thrombin and the acridine compounds.

EXPERIMENT 2.

In the manner described in Experiment 1, the periods required for the coagulation of heparinised blood plasma by a thrombin preparation with and without previous reaction with 2-ethoxy-6:9-diamino-acridine lactate were determined. 0.2 cc. of oxalated plasma from cattle, which had been freed from prothrombin by adsorption with barium sulphate, and 0.1 cc. of a heparin solution of 0.0015 per cent. strength (corresponding to 1.5 micrograms of heparin) were mixed at 37° C. with 0.3 cc. of a mixture of 1 cc. of thrombin solution, on the one hand, with 0.5 cc. of water and, on the other, with 0.5 cc. of aqueous solutions of 2-ethoxy-6:9-diamino-acridine lactate of 0.1 and 0.01 per cent. strength, respectively, and the coagulation periods were measured with a stop watch. The results are set out in the following Table:

Coagulation period without the acridine compound	Coagulation period after reaction with the solution of the acridine compound of	
	0.1 per cent. strength	0.01 per cent. strength
22 seconds	at once	14.3 seconds

The other acridine compounds used in Experiment 1 have the same effect.

As compared with the untreated thrombin solutions, the solutions obtained by the reaction of thrombin preparations with the acridine compounds have an enhanced stability. for therapeutic application, however, it is advisable to convert the preparations in known manner, for example, by freeze-drying, into dry preparations from which fresh solutions can be prepared immediately before use by addition of the appropriate quantities of water. The dry preparations are stable for practically an unlimited time.

The following Examples illustrate the invention:—

Example 1.

1 litre of a thrombin solution, obtained in known manner from oxalated plasma from cattle and containing 20 grams of dry substance, is mixed with a solution of 0.4 gram of 2 - ethoxy - 6:9 - diamino-acridine lactate in 10 cc. of water, neutralised and, if desired, centrifuged until clear and freeze-dried in known manner. 20.4 grams of a voluminous dry powder are obtained, which dissolves in water to a clear solution and, when charged into ampoules, is stable at room temperature for an unlimited time. By dissolving 0.1 gram of this product in 5 cc. of water, a solution is obtained which can be used for therapeutic purposes and has an optimum activity.

Instead of 2 - ethoxy - 6:9 - diamino-acridine lactate, the same quantity of 2 - ethoxy - 9 - [para - (γ - diethylamino-β - hydroxypropylamino)] - phenylamino-acridine trihydrochloride or 3-nitro-9-(γ-diethylamino - β - hydroxy - propyl-amino)-acridine dihydrochloride may be used.

Example 2.

1 litre of the thrombin solution used in Example 1 is mixed with a solution of 0.2 gram of 2-ethoxy-9-ethyl-amino-acridine hydrochloride in 10 cc. of water and the whole is worked up as described in that Example.

Instead of the above acridine compound, 2-ethoxy-9-amino-acridine methochloride may be used.

Example 3.

1 litre of the thrombin solution used in Example 1 is mixed with a solution of 0.1 gram of 2-ethoxy-9-(para-hydroxy-phenyl-ethylamino) acridine glycollate or 2 - ethoxy - 9 - [1^1 - phenyl - 2^1:3^1-dimethylpyrazolonyl - (4^1)] - amino-acridine hydrochloride in 10 cc. of water, and the whole is worked up to form a dry preparation as described in Example 1. A stable dry preparation is obtained, of which 0.1 gram when dissolved in 5 cc. of water, has an excellent hemostatic action.

Example 4.

Before freeze-drying a thrombin preparation made as described in Example 1, 20 grams of starch are mixed therewith to form a homogeneous mass. The resulting dry preparation has an especially good hemostatic action when applied in the dry state to a bleeding surface.

Example 5.

200 cc. of a neutralized pectin solution of 10 per cent. strength are added to a thrombin preparation made up as described in Example 2. After freeze-drying as above described, a thrombin preparation of excellent hemostatic action is obtained.

Example 6.

100 cc. of a polyvinyl pyrrolidone solution of 10 per cent. strength are added to a batch thrombin preparation made up as described in Example 3, and the whole is worked up in the usual manner.

Example 7.

200 mg. of the dry powder obtained as described in the first paragraph of Example 1 are dissolved in 5 cc. of a gelatin solution of 3 per cent. strength. A thrombin preparation is obtained which has an especially good hemostatic action when sprayed on wounds.

Example 8.

1 litre of the thrombin solution described in the first paragraph of Example 1 is concentrated *in vacuo* at an internal temperature of 5° C.—10° C. to half its weight. 500 cc. of a gelatin solution of 6 per cent. strength and a solution of 0.4 gram of 2-ethoxy-9-ethyl-amino-acridine hydrochloride in 20 cc. of water are added, and the whole is freeze-dried in the usual manner.

About 50 grams of a voluminous dry powder are obtained which has an excellent hemostatic effect when strewn on wounds.

What we claim is:—

1. A process for the manufacture of hemostatic preparations. wherein thrombin is reacted in aqueous solution with an acridine compound containing a basic substituent in the 9-position.

2. A process as claimed in claim 1, wherein the resulting solution is converted into a dry preparation by freeze-drying.

3. A process as claimed in claim 1 or 2, wherein a substance known to accelerate the action of thrombin is also incorporated in the preparation.

4. A process as claimed in claim 1, 2 or 3, wherein an aqueous thrombin solution obtained from plasma from cattle is

used.

5. A process as claimed in any one of claims 1—4, wherein the acridine compound used is 2-ethoxy-6:9-diamino-acridine lactate, 2-ethoxy-9-[para-(γ-diethylamino - β - hydroxypropylamino)-phenylamino] - acridine trihydrochloride, 3 - nitro - 9 - (γ - diethylamino - β-hydroxypropylamino) - acridine dihydrochloride, 2-ethoxy-9-ethylamino-acridine hydrochloride, 2-ethoxy-9-(para-hydroxy-phenyl-ethylamino)-acridine glycollate or 2 - ethoxy - 9 - [1^{1} - phenyl - 2^{1}:3^{1}-dimethylpyrazolonyl] - (4^{1})] - amino-acridine hydrochloride.

6. A process for the manufacture of a hemostatic preparation conducted substantially as described in any one of Examples 1—8 herein.

7. A hemostatic preparation which comprises a reaction product of thrombin with an acridine compound containing a basic substituent in the 9-position.

8. A hemostatic preparation as claimed in claim 7, which is in the form of a solution.

9. A hemostatic preparation as claimed in claim 7 which is in dry form.

10. A hemostatic preparation as claimed in claim 7, 8 or 9, which also contains a substance known to accelerate the action of thrombin.

11. A process as claimed in any one of claims 7—10, wherein the acridine compound is 2-ethoxy-6:9-diamino-acridine lactate.

12. A process as claimed in any one of claims 7—10, wherein the acridine compound is 2-ethoxy-9-[para-(γ-diethylamino - β - hydroxy - propylamino)-phenylamino]-acridine trihydrochloride.

13. A process as claimed in any one of claims 7—10, wherein the acridine compound is 3-nitro-9-(γ-diethylamino-β-hydroxypropylamino)-acridine dihydrochloride.

14. A process as claimed in any one of claims 7—10, wherein the acridine compound is 2-ethoxy-9-ethylamino-acridine hydrochloride.

15. A process as claimed in any one of claims 7—10, wherein the acridine compound is 2-ethoxy-9-amino-acridine methochloride.

16. A process as claimed in any one of claims 7—10, wherein the acridine compound is 2 - ethoxy - 9 - (para - hydroxy-phenyl-ethylamino)-acridine glycollate.

17. A process as claimed in any one of claims 7—10, wherein the acridine compound is 2-ethoxy-9-[1^{1}-phenyl-2^{1}:3^{1}-dimethylpyrazolonyl - (4^{1})] - amino-acridine hydrochloride.

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